Effects of *Astragalus membranaceus* on Energy Metabolism and Expression of CNTF Protein in Skeletal Muscle of Exercise-induced Fatigue Rats

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Abstract [**Objectives**] This study was conducted to investigate the effects of *Astragalus membranaceus* in different groups on energy metabolism and CNTF protein expression in skeletal muscle of exercise-induced fatigue rats. [**Methods**] Thirty-five clean male SD rats were randomly divided into a normal group, and low-, meddle- and high-dose groups of *A. membranaceus* aqueous solution, with 7 rats in each group. The low-dose, medium-dose and high-dose groups were given by gavage at 0.65, 1.3 and 2.6 g/kg, respectively, while the normal group and the model group were given normal food and water. The weight of rats was observed. The contents of serum urea, lactate, muscle glycogen, liver glycogen and CNTF expression were detected. [**Results**] After modeling, compared with the normal group, the serum lactate and urea contents of rats in the model group significantly increased ($P<0.01$), while the muscle glycogen content ($P<0.01$) and liver glycogen content ($P<0.05$) of the skeletal muscle significantly decreased. Compared with the model group, the low-, meddle- and high-dose groups of *A. membranaceus* significantly reduced the levels of lactate and urea in serum ($P<0.01$), while the levels of muscle glycogen and liver glycogen in the skeletal muscle significantly increased ($P<0.01$, $P<0.05$). [**Conclusions**] This study provides a good research foundation for the treatment of exercise-induced fatigue using traditional Chinese herb *A. membranaceus* in modern clinical practice.

Key words *Astragalus membranaceus*; Exercise fatigue; Energy metabolism; Skeletal muscle; Expression of CNTF protein

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Radix Astragali (*Astragalus membranaceus*) refers to dry roots of *A. membranaceus* (Fisch.) bge. var. *mongholicus* (bge.) hsiao or *A. membranaceus* (Fisch.) bge. of Leguminosae. It has the effects of invigorating qi and yang, strengthening exterior and stopping sweating, promoting diuresis and reducing swelling, promoting fluid production and nourishing blood, and promoting stagnation and relieving arthralgia[1]. *A. membranaceus* was first published in *Shen Nong's Herbal Classic* to be slightly warm in mature and sweet in taste and attributive to the spleen and lung meridians. *A. membranaceus* is a kind of qi-invigorating herb, which can tonify all the qi in the body. It was praised by the ancients as "the best of all qi-invigorating herbs". Modern pharmacological research shows that the effective components contained in *A. membranaceus* have nutritional, regulatory and protective effects on immune, circulatory, urinary and endocrine systems. Therefore, based on the principle of "invigorating the spleen and replenishing qi, benefiting qi and promoting fluid production", a rat model of exercise fatigue was established, and through the intervention of *A. membranaceus*, the effects of *A. membranaceus* on the expression of CNTF protein in the skeletal muscle of exercise fatigue rats were discussed, hoping to provide a good research foundation for the treatment of exercise-induced fatigue using traditional Chinese herb *A. membranaceus* in modern clinical practice.

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Materials and Methods

Experimental materials

Animals Clean SD rats, male, weighing 180-220 g each, were purchased under license number: Scxk (Xiang) 2019-0014 from Changsha Tianqin Biotechnology Co., Ltd.

Instruments DHG-9240A electrothermal constant-temperature blast drying oven (Shanghai Yuejin Medical Instrument Factory), HB-RO/60 ultrapure water instrument (Millipore, USA), BPH-9042 precision constant-temperature incubator (Shanghai Yiheng Scientific Instrument Co., Ltd.) and FINESSE E + paraffin microtome and real-time fluorescent quantitative PCR instrument were all purchased from Thermo Company of the United States. Universal Hood II nucleic acid protein gel imager, Mini Trans-Blot® Cellprotein wet transfer instrument and Mini Sub Cell GT protein electrophoresis instrument were all purchased from Bio-Rad Company in the United States. MF53 optical microscope was purchased from Mshot Photoelectric Technology Co., Ltd.

Drugs and reagents *A. membranaceus* was purchased from Beijing Tongrentang Guiyang Pharmacy Co., Ltd., and it was identified as the dried roots of *A. membranaceus* (Fisch.) bge. var. *mongolicus* (bge.) hsiao or *A. membranaceus* (Fisch.) bge. in Leguminosae. Lactic acid ELISA kit, urea ELISA kit, hepatic glycogen ELISA kit and muscle glycogen ELISA kit were all

purchased from Ruixin Biotechnology Co., Ltd. Eosin stain (batch number: G1002), hematoxylin stain (batch number: G1004) and PBS (batch number: G0002) were all purchased from Servicebio Technology Co., Ltd. Neutral resin (batch number: 10004160) was purchased from Sinopharm Chemical Reagent Co., Ltd..

Methods

Preparation of drug liquid First, 64 g of *A. membranaceus* was weighed and added with 8 times of water to soak the material for 30 min. Next, it was boiled with strong fire, and decocted with slow fire for 40 min. Filtration was performed to get a filtrate, and the filter residue was decocted with 6 times of water for 30 min. The two filtrates were combined and concentrated to 196 ml. The concentrate was cooled, sealed with plastic wrap and stored in a refrigerator at 4 ℃.

Grouping and administration Thirty five male SD rats were fed adaptively for 1 week, and randomly divided into a blank group, a model group, and low-, middle- and high-dose groups of *A. membranaceus* decoction, with 7 rats in each group. In the experiment, the medicated groups were given corresponding doses (0.65 g/kg for the low-dose group, 1.3 g/kg for middle-dose group and 2.6 g/kg for the high-dose group) of *A. membranaceus* decoction one hour before training, and the blank group and model group were given food and water normally.

Modeling Rats in the model group, and low-, middle- and high-dose groups of *A. membranaceus* decoction were placed in stainless steel tanks with a water temperature of (30 ± 2) ℃ and a water depth of 50 cm for swimming training. In order to prevent the rats from floating on the water, a circulating state was made at any time with wooden sticks. The administration groups received low, middle and high doses of *A. membranaceus* decoction by gavage 30 min before swimming every day. The rats swam for 1 h every day, and the dose of the decoction was adjusted according to body weight every 3 d. Those who sunk in training were exhausted, and exhausted rats could quit training early. The normal group was given food and water, and was in a quiet state. The modeling time was 4 weeks.

Sample collection Immediately after the end of the last swimming, the animals in various groups were injected intraperitoneally with 10% chloral hydrate for anesthesia. Blood was taken from the abdominal aorta and centrifuged at 3 000 r/min for 10 min, and serum was taken and stored in a −80 ℃ refrigerator. After blood collection, the skeletal muscle of the left limb and the liver tissue of rats were fixed with 4% formaldehyde, and the skeletal muscle of the right limb was frozen for later use.

Detection indexes

(1) General observation: Recording the weight of rats.

(2) Observation of pathological changes in skeletal muscle and liver tissue of exercise-induced fatigue rats treated with *A. membranaceus* by the HE staining method: The pathological tissue was detected by HE staining. The gastrocnemius muscle of the left limb fixed by 4% paraformaldehyde was subjected to dehydration with different gradients of ethanol, transparentizing, paraffin penetration, embedding, slicing, heating, deparaffinage, rehydration, washing with double distilled water for 3 times, staining, dehydration and sealing, and observed under an microscope.

(3) ELISA detection of lactate, urea, muscle glycogen and liver glycogen levels in exercise-induced fatigue rats treated with *A. membranaceus*: Lactate, urea, muscle glycogen and liver glycogen in rats were detected according to the instructions of ELISA kits. ① The kits were equilibrated at room temperature. ② Standard and sample wells were set, and 50 μl of standards with different concentrations were added to the standard wells. To sample wells, 10 μl of samples to be tested were added, and 50 μl of sample diluents were then added. ③ To each of the standard and sample wells, 100 μl of detection antibodies labeled with horseradish peroxidase were added. The plates were sealed with sealing film and incubated at 37 ℃ for 60 min. ④ After the incubation was completed, the liquid in the plates was discarded, and the plates were dried on absorbent paper. Each well was filled with a washing solution, and stood for 1 min, and the washing solution was then discarded, and the plates were dried on absorbent paper. The operation was repeated 5 times. ⑤ To each well, substrate A and B were added, 50 μl each, and incubation was then allowed in dark at 37 ℃ for 15 min. And ⑥ after incubation, 50 μl of termination solution was added to each well. The *OD* value of each well was measured at a wavelength of 450 nm within 15 min.

(4) Western blotting detection of CNTF protein expression in skeletal muscle of exercise-induced fatigue rats treated with *A. membranaceus*: Total protein was extracted first. ① The tissue ground into powder was transferred into a precooled 1.5 ml centrifugal tube. ② The cell sample in the 1.5 ml centrifugal tube was centrifuged to obtain a precipitate. The precipitate was washed with PBS and centrifuged again, and the operation was repeated twice. ③ A RIPA lysis solution was added. ④ The obtained system was oscillated for 1 min and stood on ice for 10 min, and the operation was repeated thrice to fully lyse the cells. ⑤ Centrifugation was performed at 4 ℃ and 13 000 rpm for 20 min. And ⑥ the supernatant was transferred to a precooled 1.5 ml EP tube and stored at −80 ℃.

WB detection was carried out next. ① Prepared gel was taken out from a refrigerator at 4 ℃ and put into an electrophoresis tank. ② Next, 500 μg of total protein from each sample was mixed with 5 × SDS loading buffer at a ratio of 4 ∶ 1, and the protein concentration after mixing was about 3.3 μg/μl. The obtained protein liquid was denatured by heating in a metal bath at 100 ℃ for 6 min. ③ From each denatured total protein sample, 60 μg was loaded for detection. ④ The samples were electrophoresized. ⑤ The clips were opened to keep the black side horizontal, and a sponge pad, filter paper, gel, a PVDF membrane (activated by methanol), filter paper and a sponge pad were put on it in turn. Meanwhile, the electophoresis buffer was changed into a transfer liquid. ⑥ The current was adjusted to determine the transfer time. ⑦ The membrane was taken out, labeled and

cleaned. ⑧ The primary antibodies were diluted, and incubation was carried out at 4 ℃ overnight. ⑨ Washing was carried out thrice with TBST, 10 min each time. ⑩ The secondary antibodies were diluted, and incubation was carried out at room temperature. ⑪ Washing was carried out thrice with TBST, 10 min each time. And ⑫ the products were finally exposed.

Statistical processing The experimental data were expressed by ($\bar{x} \pm s$), and statistically processed using SPSS 25.0 statistical software, including one-way ANOVA and two independent samples T-test, with $P<0.05$ or $P<0.01$ indicating statistically-significant differences.

Results and Analysis

General observation: Recording the weight of rats

From the results in Table 1, there were no significant differences in the weight of rats in each group before modeling. After modeling, compared with the normal group, the weight of rats in the model group decreased significantly ($P<0.01$). Compared with the model group, the weight of rats in the high-dose *A. membranaceus* group increased significantly ($P<0.01$), and the weights of rats in the low- and middle-dose *A. membranaceus* groups also increased to different degrees.

Table 1 Effects of different groups of *A. membranaceus* on weight of rats ($\bar{x} \pm s$, $n=7$)

Group	Dose//g/kg	Before modeling	After modeling
Normal group	–	216.286 ± 5.964	334.429 ± 8.960
Model group	–	214.571 ± 4.237	299.714 ± 9.013**
Low-dose *A. membranaceus* group	0.65	218.429 ± 4.791	337.857 ± 9.634
Middle-dose *A. membranaceus* group	1.3	218.571 ± 6.079	342.429 ± 10.228
High-dose *A. membranaceus* group	2.6	218.857 ± 4.562	357.571 ± 12.040$^{\#\#}$

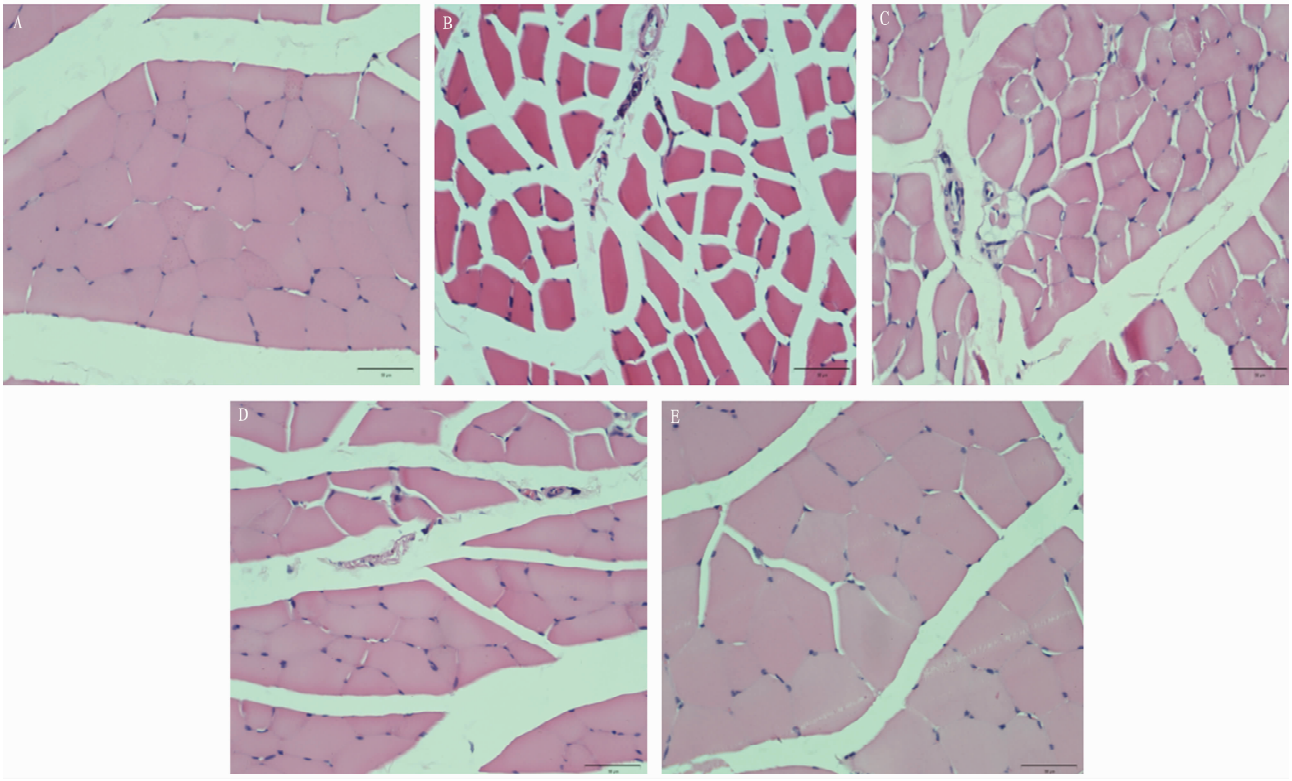
Compared with the normal group: $^{**}P<0.01$, $^{*}P<0.05$; compared with the model group: $^{\#\#}P<0.01$, $^{\#}P<0.05$.

Observation of pathological changes in skeletal muscle and liver tissue of exercise-induced fatigue rats treated with *A. membranaceus* by the HE staining method

Observation of pathological changes in skeletal muscle of exercise-induced fatigue rats treated with *A. membranaceus* by the HE staining method Normal group: Muscle fibers and nuclei were uniform in size, neatly arranged, clear in structure and evenly colored, and no obvious abnormal changes were found. Model group: The gap between muscle fibers was obviously widened, and the arrangement was loose and disordered. The muscle fibers were deformed in different sizes, and the nuclei were swollen. A small amount of inflammatory cells were infiltrated. Low-dose *A. membranaceus* group: The muscle fibers were disordered in arrangement and structure, and the gap was significantly widened. The muscle fibers were round, and irregular in shape, with different sizes. The dissolution and necrosis of muscle fibers could be observed, and the nuclei moved inward. The phenomenon of foam-like change of cytoplasm with multiple central nuclei appeared. Middle-dose *A. membranaceus* group: Muscle fibers were loosely arranged. Some of them were round and different in size, and some of them were broken and dissolved. High-dose *A. membranaceus* group: Muscle fibers and nuclei were uniform in size, neatly arranged, clear in structure and evenly colored, and no obvious abnormal changes were found. The results are shown in shown in Fig. 1.

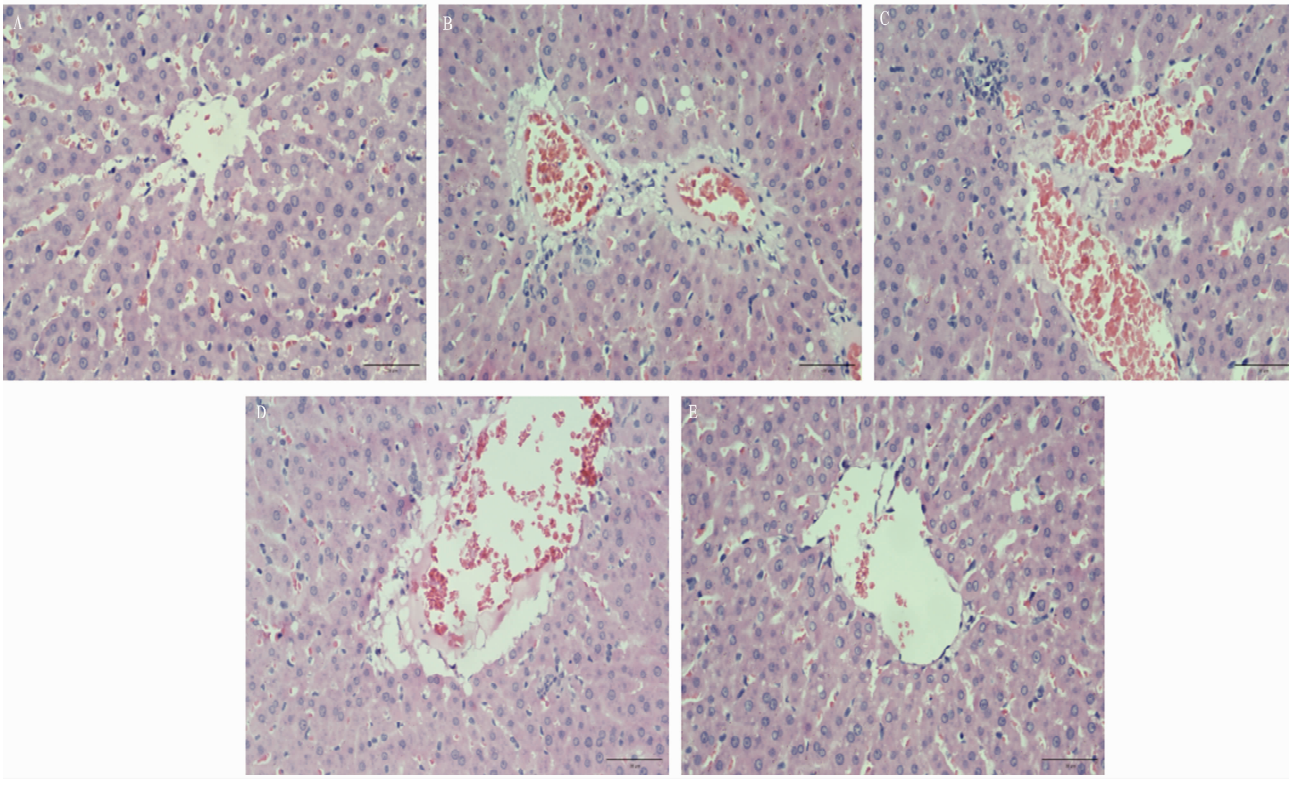
Observation of pathological changes in liver tissue of exercise-induced fatigue rats treated with *A. membranaceus* by the HE staining method Normal group: The boundary of hepatocytes was blurred, and epithelial cells of central vein fell off. The lumen of hepatic sinusoid became larger. Lymphocytes were infiltrated, and some hepatocytes were dissolved badly. The structure of hepatic cords was disordered. Model group: The hepatocytes in the portal area were tightly arranged, and there were pink exudates and lymphocytes visible in the lumen. Some hepatocytes contained fat droplets of varying sizes. Low-dose *A. membranaceus* group: Red blood cells accumulated in the lumen of the portal area, in the upper left of which was observed fragmented necrosis of lymphocytes, and punctate necrosis was observed in the hepatic sinusoid area. Hepatocellular edema was also observed with occasional lymphocytes. Middle-dose *A. membranaceus* group: The central vein was detached and ruptured, with pink exudate appearing in the lumen. There was a large amount of punctate necrosis of hepatocytes in the hepatic sinusoid area. The hepatocytes were edematous and showed blurred boundary. High-dose *A. membranaceus* group: The hepatocytes were edematous and showed blurred boundary. The epithelial cells of the central vein shed, and the lumen of hepatic sinusoid became larger. A few lymphocytes and occasional hepatocyte necrosis were observed. The results are shown in Fig. 2.

ELISA detection of lactate, urea, muscle glycogen and liver glycogen levels in exercise-induced fatigue rats treated with *A. membranaceus* The results in Table 2 showed that after modeling, compared with the normal group, the contents of lactate and urea in serum of rats in the model group increased significantly ($P<0.01$), while the contents of muscle glycogen ($P<0.01$) and liver glycogen ($P<0.05$) in the skeletal muscle decreased significantly. Compared with the model group, the contents of lactate and urea in serum of rats in the low-, middle- and high-dose groups of *A. membranaceus* significantly decreased ($P<0.01$), and the contents of muscle glycogen and hepatic glycogen in the skeletal muscle significantly increased ($P<0.01$), but the content of hepatic glycogen in the liver tissue of rats in the low-dose group of *A. membranaceus* also significantly increased ($P<0.05$).



A. Normal group ×400; B. Model group ×400; C. Low-dose *A. membranaceus* group ×400; D. Middle-dose *A. membranaceus* group ×400; E. High-dose *A. membranaceus* group ×400

Fig. 1 Effects of *A. membranaceus* on pathological changes in skeletal muscle of exercise-induced fatigue rats



A. Normal group ×400; B. Model group ×400; C. Low-dose *A. membranaceus* group ×400; D. Middle-dose *A. membranaceus* group ×400; E. High-dose *A. membranaceus* group ×400

Fig. 2 Effects of *A. membranaceus* on pathological changes in liver tissue of exercise-induced fatigue rats

Western blotting detection of CNTF protein expression in skeletal muscle of exercise-induced fatigue rats treated with *A. membranaceus* The results showed that after modeling, the expression of CNTF protein in the skeletal muscle of the model group decreased compared with that of normal group, but there was no significant difference. Compared with the model group, the expression of CNTF protein in the liver tissue of rats in the low-, medium- and high-dose groups of *A. membranaceus* increased significantly ($P<0.01$), especially in high-dose group of *A. membranaceus*. The results are shown in Table 3 and Fig. 3.

Table 2 Effects of *A. membranaceus* on lactate, urea, muscle glycogen and liver glycogen levels in exercise-induced fatigue rats ($\bar{x}\pm s$, $n=7$)

Group	Dose // g/kg	Lactate // mmol/L	Urea // mmol/L	Muscle glycogen // g/kg	Liver glycogen // g/kg
Normal group	–	4.159 ± 0.775	6.639 ± 1.179	5.629 ± 0.590	9.115 ± 1.249
Model group	–	9.457 ± 0.860**	10.256 ± 1.468**	2.995 ± 0.573**	7.084 ± 0.799*
Low-dose *A. membranaceus* group	0.65	6.777 ± 0.691$^{\#\#}$	8.441 ± 0.748$^{\#\#}$	6.482 ± 1.196$^{\#\#}$	8.851 ± 1.117$^{\#}$
Middle-dose *A. membranaceus* group	1.3	5.238 ± 0.855$^{\#\#}$	7.934 ± 0.694$^{\#\#}$	7.039 ± 0.711$^{\#\#}$	11.704 ± 1.518$^{\#\#}$
High-dose *A. membranaceus* group	2.6	4.698 ± 0.871$^{\#\#}$	6.824 ± 0.773$^{\#\#}$	8.668 ± 1.236$^{\#\#}$	14.606 ± 2.185$^{\#\#}$

Compared with the normal group: $^{**}P<0.01$, $^{*}P<0.05$; compared with the model group: $^{\#\#}P<0.01$, $^{\#}P<0.05$.

Table 3 Western blotting detection of CNTF protein expression in skeletal muscle of exercise-induced fatigue rats treated with *A. membranaceus* ($\bar{x}\pm s$, $n=7$)

Group	Dose // g/kg	CNTF protein expression
Normal group	–	0.404 ± 0.050
Model group	–	0.372 ± 0.056
Low-dose *A. membranaceus* group	0.65	0.586 ± 0.047$^{\#\#}$
Middle-dose *A. membranaceus* group	1.3	0.642 ± 0.071$^{\#\#}$
High-dose *A. membranaceus* group	2.6	0.863 ± 0.067$^{\#\#}$

Compared with the normal group: $^{**}P<0.01$, $^{*}P<0.05$; compared with the model group: $^{\#\#}P<0.01$, $^{\#}P<0.05$.

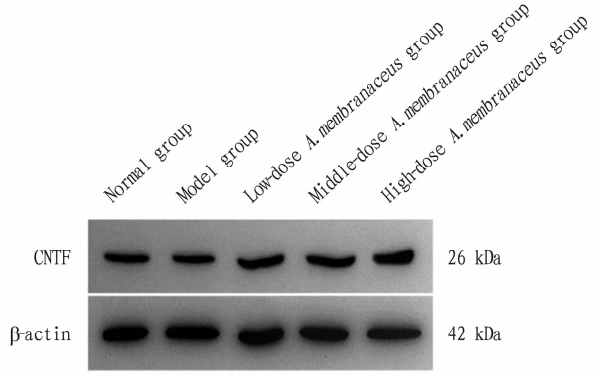


Fig. 3 Effects of *A. membranaceus* on the expression of CNTF protein in skeletal muscle of exercise-induced fatigue rats

Conclusions and Discussion

Exercise-induced fatigue refers to the decline of physical function caused by excessive exercise, which is a normal physiological reaction when exercise reaches a certain stage. It is a comprehensive external manifestation of various internal complex changes in the body (complex changes of multiple tissues, organs, systems and levels), belonging to the subclinical state in state medicine[2]. When the body undergoes short-term and high-intensity exercise, the energy metabolism mode changes, transitioning from aerobic energy supply to anaerobic glycolysis energy supply. During prolonged and high-intensity exercise, energy depletion, accumulation of metabolic products, disorder of ion metabolism and excessive generation of free radicals lead to exercise-induced fatigue[3-5].

Compared with fatigue, exhaustion refers to the state where the body does not recover well after fatigue, and the fatigue state continues to accumulate and deepen, gradually developing into an injury state. When the body is completely unable to maintain movement, it will present a state of exhaustion[6]. According to the mechanism of exercise-induced fatigue, prevention and treatment often follow the principles of strengthening the spleen and kidney, supplementing qi and blood, and relaxing tendons and promoting blood circulation[7].

The indicator that reflects the development level of bones and muscles in the body is weight. During sports training, changes in body weight reflect the degree of impact of training on the body and the body's adaptation to training[8]. The change in weight mainly comes from increases in the body's bones, muscles and fat, excessive fat accumulation, increased bone density, and increased muscle content, all of which can directly affect the change in weight. The results of this study showed that there were no significant differences in the weight of rats among various groups before modeling; after modeling, the weight of rats in the model group decreased significantly; and compared with the model group, the high-dose *A. membranaceus* group showed a significantly-increased weight of rats, and the low-dose and middle-dose groups of *A. membranaceus* also exhibited varying degrees of increase in weight. It indicated that the administration of *A. membranaceus* liquid had a certain alleviating effect on the body damage caused by long-term exercise, and the effect showed a significant increase with the increase of dosage.

Sports fatigue is a common phenomenon in sports training, and liver glycogen, muscle glycogen, blood lactate and urea nitrogen can all reflect the degree of fatigue in the body[9]. Lactate is the final product of glycolysis. During exercise, the anaerobic glycolysis process of skeletal muscle glycogen begins and strengthens. While the glycolysis reaction generates energy, a large amount of lactate is also generated, and it rapidly increases and accumulates in the blood and muscles. In order to maintain acid-base balance, lactate gradually diffuses into the blood, leading to an increase in the content of lactate in the blood, thereby affecting the balance of the internal environment and reducing the body's exercise ability. Therefore, the rapid increase in blood lactate level in the body is

the direct cause of exercise-induced fatigue. After vigorous exercise, the accumulation level of blood lactate directly reflects the degree of fatigue in the body[10].

Urea is the final product of protein and amino acid catabolism in the body. The content of serum urea can reflect the intensity of protein or amino acid catabolism in the body. When the body receives a certain degree of exercise, the load will increase, and the catabolism level of proteins and amino acids will increase, resulting in a higher urea content in the blood[11-13]. Therefore, the level of blood urea can directly reflect the decomposition of proteins in the body, thereby determining the degree of fatigue in the body. The experimental results showed that compared with the normal group, the serum lactate and urea contents of rats in the model group significantly increased. After administering low, medium, and high doses of *A. membranaceus*, the serum lactate and urea contents of the rats were significantly reduced. It indicated that administration of *A. membranaceus* liquid could reduce the accumulation of blood lactate, which might be achieved by increasing the activity of certain enzymes that regulate lactate metabolism. For urea, it might be because *A. membranaceus* contained a lot of energy substances such as polysaccharides and amino acids, which increased the glycogen storage in rats, reduced the decomposition and utilization of proteins and amino acids during exercise, and then inhibited the production of urea.

Sugar is the main component of the body's energy supply, and it is also the most important energy substance and the material basis for improving the body's sports ability. Sugar in the body mainly exists in the form of glycogen and glucose[14-15]. Glycogen can be divided into muscle glycogen and liver glycogen. Muscle glycogen is a reserve energy source that can be used at any time in the skeletal muscle, and its reserve is positively related to exercise endurance. Liver glycogen maintains the relative stability of blood sugar, and with the extension of exercise time, liver glycogen will be consumed too much, resulting in insufficient energy supply for exercise muscles and peripheral fatigue[16]. Therefore, the content of glycogen has a great correlation with the degree of exercise fatigue. According to research reports by Feng *et al.*[17], when the reserves of liver and muscle glycogen in the body are low, the output power of muscles also decreases, leading to fatigue. Therefore, increasing the reserves of liver and muscle glycogen and reducing loss is the best way to maintain stable blood sugar concentration and delay fatigue. More energy substances in the body enable exercise of greater intensity and for longer time, and can effectively extend the body's exercise time.

The results of this study showed that after modeling, the contents of muscle glycogen and liver glycogen in the skeletal muscle significantly decreased; and after administration, the contents of muscle glycogen and liver glycogen significantly increased. It indicated that long-term exhaustive exercise in rats led to a decrease in liver glycogen and muscle glycogen reserves, and supplementing *A. membranaceus* aqueous solution could promote glucose metabolism, increase glycogen reserves, reduce consumption, and timely decompose liver glycogen to supplement blood sugar, which ensured the oxidative energy supply of exercise muscles, improved fatigue resistance. A dose-dependent phenomenon was observed, so rats could engage in exercise of higher intensity and for longer duration. From the pathological changes in the skeletal muscle and liver tissue of exercise-induced fatigue rats treated with *A. membranaceus* solution, it could be concluded that with the increase of dosage, the pathological morphology of skeletal muscle injury and liver injury showed an upward trend in recovery.

CNTF is a known neurotrophic factor, which has a nutritional effect on motor nerves. At present, it has been found that CNTF plays a direct nutritional role in muscles, is a dual nutritional factor of nerves and muscles, and can maintain the shape and function of normal skeletal muscle[18], so it plays a great role in the process of exercise fatigue and injury recovery[19]. The experimental results showed that after modeling, the expression of CNTF protein in the skeletal muscle of rats decreased compared with the normal group, but there was no significant difference; and compared with the model group, the expression of CNTF protein in the liver tissue of rats in the administration groups increased significantly, especially in the high-dose group of *A. membranaceus*. It indicated that the relative expression of protein in aqueous solution of *A. membranaceus* increased in dose, which indicated that the relative expression of CNTF was up-regulated with the dose of *A. membranaceus* aqueous solution, and *A. membranaceus* indirectly played a role in maintaining normal morphology, function and nutritive function of skeletal muscle.

A. membranaceus is an important herb for invigorating the middle energizer and benefiting qi. Related research shows that *A. membranaceus* can reduce the influence of high-intensity exercise, improve the energy metabolism of the body, increase glycogen storage during exercise, and enhance the anti-fatigue ability of rats, suggesting that *A. membranaceus* has a good development prospect in preventing exercise fatigue, which embodies its remarkable characteristics of multiple targets and multiple pathways.

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(Continued on page 29)

urine protein and alleviating renal tissue damage.

In summary, this study preliminarily confirmed that Wuling Powder had a protective effect on the kidneys of NS mice, and alleviated renal pathological damage. Its mechanism might be related to the inhibition of the Notch pathway, providing a theory for later development and utilization of Wuling Powder and drug development.

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